

INFLUENCE OF SOIL CHARACTERISTICS ON BIOREMEDIATION OF HYDROCARBONS CONTAMINATED SOIL

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ABSTRACT

The growth of industrialization and population ultimately results in the pollution of soil, water, air and other components of the environment. The soil is a medium for the growth of plants and microorganisms; as such soil is a major component of a bioremediation system. Hence, research on bioremediation should take into account the influence of physical and chemical characteristics of the contaminated soil. The bioremediation of hydrocarbon contaminated soil has not received much attention of researchers. Thus, a series of laboratory experiments were conducted on three different types of soil, with the aim of developing a new method of bioremediation, in addition to other controlling factors, taking into account the physical and chemical characteristics of soil for remediation of hydrocarbon contaminated soil, and to identify the most suitable soil type, which is potential for bioremediation using indigenous microorganisms.

Key Words : Soil, Bioremediation, Hydrocarbons, Contamination, Micro organisms

INTRODUCTION

Oil pollution adversely affects the soil ecosystem through adsorption to soil particles, makes an imbalance of carbon, nitrogen and phosphorus that might be unavailable for microbial use and these cause a delay in the natural rehabilitation of oil polluted soils. The various soil treatments have been used in bioremediation strategies to improve the process. These include

surfactants, alternate carbon substrates and organic and inorganic nitrogen and phosphorus. The effectiveness of these treatments depends on the heterogeneity of soils and oil samples as well as possible interactions between the soil and its constituents. The influence and effectiveness of properties in any soil, therefore needs to be evaluated on case specific basis¹.

Soil has many ecological and socioeconomic functions including the

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capacity to remove contaminants from the soil environment, soil can be considered as a limited and non-renewable resource on a 50–100 year timescale². The surface soil characteristics are influenced by vegetation, biota, topography and human activities. As a consequence, there is a wide diversity of soil types and its potential for contaminant degradation differs significantly.

Many activities are known to cause soil contamination. The Contamination of soils results in most cases from the careless use of chemicals or ignorance. The typical activities that have caused contamination of soils by hydrocarbons are petroleum underground storage tanks, distribution facilities, industrial operations, vehicle garages, service stations, and bus depots. The most common harmful substances causing the contamination of soils are oil products, heavy metals, polyaromatic hydrocarbons, polychlorinated biphenyls, chlorophenols, and pesticides.

The soil chemistry is equally important in developing a biodegradation potential for contaminated soil. For instance, the soil pH should be adjusted to within the range 6–8 to enhance microbial activity¹. The levels of nitrogen and phosphorus in the soil may also be very critical as these may limit the biodegradation rates. The levels of N, P, K, Fe, Mg and C etc., and porosity, permeability, grain size distribution, uniformity coefficient, coefficient of curvature etc., which determine the soil physico-chemical properties along with the pH, could limit the metabolism of the existing microorganisms capable of degrading hydrocarbons in the soil environment. Application of extra

nutrients may be required for developing a feasible bioremediation method². Retention of organic contaminants on coarser soil fractions and aquifer material after soil washing or flushing may be influenced by several factors other than particle surface area, including the hydrophobicity of the contaminant, the properties of the washing medium, and the characteristics of the soil particles. Extremely hydrophobic contaminants such as four and five ringed polynuclear aromatic hydrocarbons (PAHs) and high molecular weight alkanes, both constituents of petroleum products, may not readily partition from a soil surface into an aqueous wash fluid. The use of surface active additives that enhance the mobility of such contaminants in the washing fluid often has a large impact on the effectiveness of soil washing and flushing^{1,2}. Similarly, the addition of extraction or chelating agents and pH adjustments can significantly improve contaminant removals. The use of such additives, however, results in the production of more complex spent wash fluid that is difficult to treat or dispose.

Soil organic matter (SOM) content has been implicated as the primary factor governing the interactions between organic contaminants and soil particles^{2,5}. Slow rates of desorption or mass transfer of contaminants from SOM matrices can render the soil washing process uneconomical for coarser material that may have large amounts of organic carbon. Mineral attributes of the soil or aquifer material, such as the presence of surface metal oxides, may also exert significant control on the extent to which SOM or organic contaminants adhere to the

mineral domain of the soil particle³. Oil is a complex mixture of thousands of hydrocarbons and hydrocarbon compounds, including heavy metals. Although the toxicity of each individual component is known, the toxicity of complex mixtures such as crude oils and refined products is extremely difficult to assess, because the chemical composition of each crude oil and petroleum product varies significantly, and can have diverse effects on different organisms within the same ecosystem. These differences in toxic effects are due to qualitative, and compositional differences in the various products, as well as concentration differences of the chemical constituents⁴.

Oils may cause serious problems when spilled on soil or on water. The daily use of hydrocarbons in vehicles and industries contaminates the soil. Some of these hydrocarbon emissions are carried by the air into the soil, or are carried by water where they can clog up soil pores, usually close to the source of the contamination. Hydrocarbons are toxic to plants and animals

Bioremediation is the enhancement of soil organisms such as fungi, bacteria and plant to break down hydrocarbon and organic contaminants. It involves the application of organisms and nutrients such as phosphate and nitrogen to the contaminated soil. Nitrate and phosphate supplements enhance biodegradation of oil^{3,6}. Bioremediation involves the transformation of complex or simple chemical compounds into nonhazardous forms by biological agents resulting in materials of a higher nutritive value or simply reducing the final bulk of the product.

This then gives rise to a variety of products most of which will be much more water-soluble than the parent hydrocarbon. White-rot fungi have been known for their ability to degrade lignin, a non repeating structural polymer found in woody plant and this ability enables them to degrade xenobiotic pollutants *Pleurotus tuberregium* (a white-rot fungus) has been reported to ameliorate oil polluted soil and the resulting soil sample supported germination and seedling of *Vigna unguiculata*. Many studies have reported the use of *Pleurotus* species in bioremediation exercises reported the ability of *Lentinus subnudus* to mineralize soil contaminated with various concentrations of crude oil^{5,8}.

Biodegradation occurs in the environment because living things (earthworms, bacteria and fungi) are actively breaking down organic substances, including many pollutants. Microorganisms are especially important in the biodegradation of pollutants. The existing organic molecules provide carbon atoms, which are used to build biological compounds, such as carbohydrates and proteins. This is a multistep process in which the large organic molecules are broken down (hydrolyzed) either inside or outside bacteria⁷.

Bacteria: Some bacteria grow and reproduce only when oxygen is present. They use the oxygen for the process of aerobic biodegradation. When oxygen is not present in an anaerobic environment (like in deep landfill sites) some bacteria remove chlorine from harmful chlorine-containing compounds, such as PCB's (polychlorinated

biphenyls - human made oils used in electrical equipment), by replacing them with hydrogen atoms which can then be used as food for the bacteria⁸.

Factors Affecting Biodegradation: During the winter, biodegradation is slow, because temperature is one factor that affects the rate of biodegradation. Other factors include soil moisture, pH, oxygen supply and nutrient availability. Bioreactors are a new technology that speeds up the rate of biodegradation. Biodegradation by adding water, planting vegetation also encourages faster biodegradation because the populations of bacteria and fungi are larger around plant roots and this higher level means more microbial activity³.

The evaluation of biodegradation and of the necessity and level of remediation requires measurements of physical, chemical and biological criteria of soil quality.

Major soil physical characteristics that may influence the bioremediation process are porosity, bulk density and air permeability. The permeability determines the rate of transfer of electron acceptors to the contaminated soil. It is believed that the reduction of permeability because of microbial biofilms in the soil macrovoids, as well as in the smaller pores of the soil matrix (microvoids), is a major hurdle in managing in situ bioremediation. This problem may be more acute at the point of nutrient application or injection.

Estimation of soil physico-chemical properties :

Though at present, details are not known about the influence of soil type on

biodegradation kinetics, it is likely that the highly sorptive surfaces of some clay and organic matter fractions limit the bioavailability of petroleum hydrocarbons to soil microorganisms⁸. This may be especially the case for intensely weathered soils where the contaminants have had time to migrate into the micropores, which are less accessible to microbial attack. In general, bioavailability of hydrocarbons declines with ageing. The rate and extent of sequestration as measured by the extent of mineralisation of phenanthrene by an added bacterium has been shown to be appreciable in soil samples with more than 2% organic carbon¹⁰. In other words, soils from various sources and locations exhibit differences in both rate and extent of sequestration. We have not conducted a study to determine the levels of sequestration at Borhola oil fields given the high levels of contaminant concentration in the field, it is expected that there should be significant levels of bioavailable contaminants. In the case of intensely weathered soils, the kinetics are not limited by the number of hydrocarbon degraders or the intrinsic petroleum hydrocarbon biodegradability, but rather by mass transport (desorption, diffusion and convection) phenomena. It is known that the biodegradation rates are affected by the fraction of fines (0.075 mm) in the soil. Soil characterised by more than 10% fines exhibited lower biodegradation rates and the extent of bioremediation during land treatment was lower than that of soils with smaller fines fractions, i.e. 10%⁸. The increased sorptive surface area of soil with larger fines fractions may affect the

bioavailability of certain hydrocarbon contaminants.

Estimation of soil microbiological properties :

The soil microbial properties are, perhaps, the vital factors determining natural biodegradability. Microbial characterization of soil includes enumeration of total microbes and contaminant-specific degraders. Soils usually contain large numbers of native or indigenous microorganisms that are able to degrade petroleum hydrocarbons. Microbial inhibition may occur in the presence of high salt concentration and heavy metals, i.e. Ni, Cr, Pd, Cd, As, etc⁹. In addition, hydrocarbon levels higher than 10% wt. are associated with varying degrees of inhibitory effects on soil microbes. Total microbial content was determined by the Agar Plate Method for Total Microbial Count as described in the literature¹⁰. The total microbial count was compared with an estimate of the population that will degrade the contaminant. The preliminary screening of the contaminant specific degraders was performed in a medium.

MATERIAL AND METHODS

Soil characterization

The three types of soil were selected and characterized before pollution with the oil. Particle size distribution was done by sieve analysis as per IS-2720-1965, the permeability was determined by both constant head and falling head tests. Porosity was determined by compaction test. Moisture was determined by gravimetric analysis. pH was determined by potentiometric method, total organic

carbon was determined by wet combustion method and total nitrogen was determined by alkaline permanganate method. Available phosphorous was determined by Olsen extraction method. The potassium was determined by flame photometry. Soil microbial population was estimated by the ten-fold serial dilution method, Population of total heterotrophic bacteria was estimated using nutrient agar (Oxoid) and potato dextrose agar respectively. The physico-chemical and microbiological characteristics of the three different types of soil are presented in **Table 1**.

The simulated contaminated soil was prepared for the above three types of soil with an oil loading rate of 40kg/sqm. And this was allowed for acclimatization of indigenous microorganisms to the polluted environment for about 20 days in the ambient environment. Then each type of simulated contaminated soil was mixed thoroughly so as to get homogeneity and filled in three different cubical reactors of dimension 30cm*30cm* 23cm. The initial microbial population was estimated by serial dilution method, and the initial total petroleum hydrocarbons (TPH) were determined gravimetric analysis. Then, the microbial growth and reduction in TPH was determined after every week for about 13 weeks in each of the three reactors the results are presented in **Table 2**.

RESULTS AND DISCUSSION

The three types of soils were selected from three different sites (A, B and C) and

their characteristics were determined as per preparing simulated contaminated soil. The standard procedure before and after results are tabulated in the following Table 1.

Table 1 : Soil properties before and two weeks after preparing () simulated contaminated soil**

Sl.No.	Physico-Chemical and microbial properties	Type A soil a b**		Type B Soil a b**		Type C Soil a b**	
1	pH	6.78	6.27	6.96	7.04	6.58	6.82
2	Temperature °C	30.5	31.3	31	31.6	31	30.4
3	Moisture (%)	4.99	6.64	5.68	7.83	6.82	8.29
4	Porosity (%)	24	-	19	-	16	-
5	Permeability (m/hr)	0.0433	-	0.0643	-	0.0735	-
Grain size distribution (%)							
6	Sand clay , silt, Uniformity coefficient(Cu), Coefficient of curvature(Cc)	76	-	68	-	62	-
		18	-	21	-	26	-
		6	-	11	-	12	-
		7.9	-	5.6	-	4.1	-
		1	-	0.93	-	0.78	-
7	Total organic carbon (%)	1.26	1.21	1.22	1.13	0.74	0.61
8	Nitrogen (mg/gm)	81.9	71.4	78.1	58.2	76.8	60.2
9	Phosphorous (mg/gm)	5.27	3.01	3.18	2.88	5.84	4.56
10	Potassium (mg/gm)	3.6	3.03	0.76	3.01	0.82	0.73
Microbial count (cfu/gm of soil)							
11	Bacteria	2.36x10 ⁶	2.48x10 ⁶	3.86x10 ⁶	3.89x10 ⁶	1.84x10 ⁶	1.93x10 ⁶
		1.48x10 ⁶	1.55x10 ⁶	2.87x10 ⁵	2.93x10 ⁵	1.32x10 ⁵	1.69x10 ⁵
		0.97x10 ⁴	1.13x10 ⁴	0.56x10 ⁴	0.84x10 ⁴	0.47x10 ⁴	0.53x10 ⁴
	Actinomycetis						

Note : 'a' refers to soil characteristics before contaminating

'b**' refers to soil characteristics two weeks after preparing simulated contaminated soil.

'-' indicates values not determined

The simulated contaminated soils are then filled into three different cubical reactors and were kept in the ambient environment for biodegradation. For each of the simulated

contaminated soil (A, B, and C) the TPH removal and the microbial population growth were determined for about 13 weeks and are tabulated in the following **Table 2**.

Table 2 : Variation of Total petroleum Hydrocarbons (TPH) and bacterial population in each of soil type at the end of successive weeks.

a. Type A Soil:

Time (Weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13
TPH (%)	33.3	33.05	32.72	31.83	31.11	30.89	29.78	28.87	27.26	25.8	24.91	23.14	20.98
Bacterial population ($\times 10^6$ cfu/g of soil)	2.43	2.48	2.56	2.64	2.69	2.72	2.77	2.83	2.93	2.96	3.49	5.89	6.17

b.Type B Soil:

Time (Weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13
TPH(%)	32.85	32.54	32.32	32.17	31.05	31.09	30.81	30.75	30.62	30.47	30.14	29.48	29.2
Bacterial population ($\times 10^6$ cfu/g of soil)	3.80	3.89	3.81	3.96	3.99	4.02	4.06	4.08	4.14	4.19	4.2	4.28	4.3

c.Type C Soil:

Time (Weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13
TPH(%)	35.28	35.17	35.06	34.82	34.76	34.53	34.21	34.17	33.94	33.73	33.46	33.26	33.14
Bacterial population ($\times 10^6$ cfu/g of soil)	1.88	1.93	1.91	1.92	1.98	2.14	2.19	2.27	2.29	2.41	2.67	2.71	2.81

From the above table, it is observed that the type A soil can degrade the TPH to the extent of 37%, where as Type B and type C soil can degrade TPH to the extent of 11.11% and 6.07% respectively. it is also observed that the bacterial growth is significant in type A soil compared to the Type B and Type C soil.

From the **Table 1.** it is observed that, the type A soil exhibits better porosity and lesser permeability which can hold organic contaminants in its pore space for better biodegradation compared to type B and type C soil. Further it is also observed from the grain size distribution analysis of type A soil, the soil can be grouped under the well graded sand with 76% sand, 18% clay, 6% silt, uniformity coefficient 7.9, coefficient of curvature 1, where as the type B and type C soils are not well graded soils with more than 10% of silt content which cannot hold the organics since these soils have lesser porosity and higher permeability. Also the chemical properties of the type A soil are favoring for better microbial growth compared to type B and type C soil.

CONCLUSION

On observing the result, it can be concluded that, 1. the soil characteristics have influence on the bioremediation potential of the soil. 2. the Type A soil has better bacterial growth as well as removal of Total petroleum hydrocarbons (TPH). Hence it can be concluded that the type A soil has better potential for biodegradation of petroleum hydrocarbons

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